



---

Year: 2019

---

## Topical resiquimod dosing regimens in patients with multiple actinic keratoses: a multicentre, partly placebo-controlled, double-blind clinical trial

Stockfleth, E ; Hofbauer, G F L ; Reinhold, U ; Popp, G ; Hengge, U R ; Szeimies, R M ; Brünig, H ; Anliker, M ; Hunger, T ; Dummer, R ; Ulrich, C ; Kenzelmann, R ; Surber, C ; French, L E

**Abstract:** BACKGROUND: Topical immune response modifiers are established for actinic keratosis (AK) treatment and efforts are underway to make further improvements to their efficacy and safety. OBJECTIVES: To investigate the optimal dosing regimens of the Toll-like receptor 7/8 agonist resiquimod in terms of efficacy, safety and tolerability. METHODS: In a multicentre, partly placebo-controlled, double-blind clinical trial, we randomized 217 patients with AK lesions to 0·03% resiquimod gel once-daily application three times per week for 4 weeks or seven times within 2 weeks or five times for 1 week (arms 1/2/3) followed by a treatment-free interval of 8 weeks and one repetition of the cycle. In two additional arms (arms 4/5), patients applied either resiquimod gel 0·01% or 0·03% three times per week up to a biological end point defined by skin erosion or for a maximum duration of 8 weeks. Clearance was assessed clinically and histologically. RESULTS: Complete clinical clearance ranged from 56% to 85% with the highest rate observed in arm 2. Resiquimod 0·03% gel was more effective than 0·01% gel. Clearance rates in arms 1/2/3 were comparable and higher than with placebo and were reached with 24, 14 and 10 gel applications, respectively. Overall, 128 patients (59%) experienced treatment-related adverse reactions. CONCLUSIONS: Resiquimod 0·03% gel is more effective than 0·01% gel. From the perspectives of safety and tolerability, the lower concentration and shorter duration are preferable. The clinical response in arms 2/3 was reached with fewer gel applications. The dosing regimens that used the biological end point (arms 4/5) proved equally efficacious as predefined treatment durations and may therefore be suitable for personalized AK treatment.

DOI: <https://doi.org/10.1111/bjd.17124>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-160017>

Journal Article

Accepted Version

Originally published at:

Stockfleth, E; Hofbauer, G F L; Reinhold, U; Popp, G; Hengge, U R; Szeimies, R M; Brünig, H; Anliker, M; Hunger, T; Dummer, R; Ulrich, C; Kenzelmann, R; Surber, C; French, L E (2019). Topical resiquimod dosing regimens in patients with multiple actinic keratoses: a multicentre, partly placebo-controlled, double-blind clinical trial. *British Journal of Dermatology*, 180(2):297-305.

DOI: <https://doi.org/10.1111/bjd.17124>

# Topical resiquimod dosing regimens in patients with multiple actinic keratosis: a multi-centre, partly placebo-controlled, double-blind, clinical trial

Stockfleth E<sup>1</sup>, Hofbauer GFL<sup>2</sup>, Reinhold U<sup>3</sup>, Popp G<sup>4</sup>, Hengge UR<sup>5</sup>, Szeimies RM<sup>6</sup>, Brünig H<sup>7</sup>, Anliker M<sup>8</sup>, Hunger T<sup>9</sup>, Dummer R<sup>2</sup>, Ulrich C<sup>10</sup>, Kenzelmann R<sup>11</sup>, Surber C<sup>2,12,13</sup>, French LE<sup>2,13</sup>

- 1 Universitätshautklinik, St. Josef-Hospital, Gudrunstrasse 56, 44791 Bochum, Germany
- 2 Dermatologische Klinik, Universitätsspital Zürich, Gloriastrasse 31, 8091 Zürich, Switzerland
- 3 Medizinisches Zentrum Bonn-Friedensplatz, Fachbereich Dermatologie, Allergologie, Dermatologische Onkologie, Friedensplatz 16, 53111 Bonn, Germany
- 4 Licca Clinical Research Institute, Hofackerstrasse 19, 86179 Augsburg, Germany
- 5 Hautzentrum Prof. Hengge, Immermannstrasse 10, 40210 Düsseldorf, Germany
- 6 Klinikum Vest GmbH, Knappschaftskrankenhaus, Abteilung Dermatologie, Dorstener Strasse 151, 45657 Recklinghausen, Germany
- 7 DERMAKIEL, Allergie und Hautzentrum, Schönberger Strasse 72-74, 24148 Kiel, Germany
- 8 Dermatologie / Allergologie, Kantonsspital St. Gallen, Roschacher Strasse 95, 9007 St. Gallen, Switzerland
- 9 Dermatologische Klinik, Inselspital, Universitätsspital Bern, Freiburgerstrasse 3, 3010 Bern, Switzerland
- 10 Hauttumorzentrum Charité (HTCC), Klinik für Dermatologie, Venerologie und Allergologie, Campus Charité Mitte, Charité – Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany
- 11 Galderma Spirig Pharma AG, Froschackerstrasse 6, 4622 Egerkingen, Switzerland
- 12 Dermatologische Klinik, Universitätsspital Basel, Petersgraben 4, 4031 Basel, Switzerland
- 13 Corresponding author

Corresponding author:  
Prof. Dr. phil. nat. Christian Surber  
Universitätsspital Zürich  
Department of Dermatology  
Gloriastrasse 31  
CH-8091 Zürich, Switzerland  
E: [christian.surber@unibas.ch](mailto:christian.surber@unibas.ch)

The trial was funded by Galderma Spirig Pharma AG, Switzerland  
The trial has been registered at ClinicalTrials.gov with the number NCT01583816

Key words:  
Actinic keratosis, resiquimod, immune response modifier, immunotherapy, toll-like receptor

**ABSTRACT**

Topical immune-response modifiers are established for actinic keratosis (AK) treatment, and efforts are underway to further improve their efficacy and safety. We investigated the optimal dosing regimens of the TLR7/8 agonist resiquimod in terms of efficacy, safety, and tolerability.

In a multi-centre, partly placebo-controlled (double-blind), clinical trial, we randomized 217 patients with AK-lesions to 0.03% resiquimod gel once daily application 3 times weekly for 4 weeks or 7 times within 2 weeks or 5 times in 1 week (Arms1/2/3) followed by a treatment-free interval of 8 weeks and one repetition of the cycle. In two additional arms (Arms4/5), patients applied either resiquimod gel 0.01% or 0.03% 3 times weekly up to a biological end-point defined by skin erosion or for a maximum duration of 8 weeks. Clearance was assessed clinically and histologically.

Complete clinical clearance ranged from 56% to 85% with the highest rate observed in Arm2. Resiquimod 0.03% gel was more effective than 0.01% gel. Clearance rates in Arms1/2/3 were comparable and higher than with placebo and were reached with 24, 14 and 10 gel applications. 128 (59%) patients experienced treatment-related adverse reactions.

Resiquimod 0.03% gel is more effective than 0.01%. The lower concentration and shorter duration are preferable from safety and tolerability perspectives. The clinical response in Arms2/3 was reached with fewer gel applications. The dosing regimens using the biological end-point (Arms4/5) proved equally efficacious as predefined treatment durations and may therefore be suitable for personalized AK treatment.

68 **What's already known about this topic?**

- 69       • Actinic keratosis (AK) occurs in sun-exposed skin areas and may progress to  
70       squamous cell carcinoma - if left untreated.  
71       • AK-treatments are often lengthy and demands high patient commitments.  
72

73 **What does this study add?**

- 74       • Resiquimod gel 0.03/0.01% was effective as AK-treatment on balding scalp,  
75       forehead, or face.  
76       • Reduction of total number of gel applications (24>10) gave comparable clearance  
77       rates and may therefore simplify AK-treatments.  
78       • Dosing regimens using the biological end-point of erosion proved equally  
79       efficacious as predefined treatment duration and may therefore be suitable for  
80       personalized AK treatment.  
81

## INTRODUCTION

Actinic keratosis (AK) occur predominantly on sun-exposed skin areas, appearing as irregularly shaped, scaly, and erythematous macules<sup>1,2</sup>. The incidence rate of AK is increasing worldwide<sup>3</sup>, with fair skinned individuals living in sunny climates<sup>2</sup>. Men are more frequently affected than women<sup>4</sup>, and prevalence increases with age<sup>5</sup> with rates ranging from 11% to 26%<sup>2,6</sup>.

AK-lesions are seen as part of a continuum along the path to the development of squamous cell carcinomas (SCC)<sup>7</sup>. The rate for AK transformation to SCC is highly variable ranging from 0.1 to 10% or more<sup>8-11</sup>. Therefore, most guidelines recommend AK to be treated<sup>2</sup>. In recent years, topical therapies have become the preferred AK therapy<sup>1,12,3</sup>. Although the immune-response modifier (IRM) imiquimod is an established treatment for AK, efforts are underway to increase efficacy, to shorten treatment duration and to reduce side effects for achieving an optimal AK treatment. Resiquimod is an IRM, which based on its pharmacodynamic profile may potentially achieve greater efficacy than imiquimod<sup>13</sup>. Resiquimod activates myeloid dendritic cells in addition to plasmacytoid cells and induces more interleukin-12 and tumour necrosis factor than imiquimod<sup>3,14,15</sup>.

A previous phase II study investigated the safety and efficacy of topical resiquimod gel in the treatment of AK-lesions<sup>14</sup>. Resiquimod gel in concentrations of 0.01%, 0.03%, 0.06% or 0.1% were applied 3x/week for 4 weeks in 1 or 2 treatment cycles. The efficacy was high in all treatment groups, with overall complete clearance rates ranging from 77.1%-90.3%. Drug-related influenza-like symptoms were observed primarily at the higher concentrations. Treatment with 0.01% resiquimod gel demonstrated the best tolerability and provided the widest therapeutic window with an overall complete clearance rate of 77% and a severe erythema rate of 17%<sup>14</sup>.

Against this background our primary objectives were to define the concentration and dosing schedule of resiquimod gel formulations at which complete clearance occurred, or at which a biological endpoint (clinical manifestation of skin erosion, i.e., the skin appears reddened, erosive, and weeping) was achieved with subsequent complete clearance. A central element of this investigation was to reduce – compared to previous trials<sup>14,16</sup> – the total number of gel applications during the treatment cycle and evaluate the suitability of using a biological endpoint to terminate treatment instead of a predefined treatment duration. The secondary objectives were efficacy, local tolerability and safety of the various concentrations and dosing schedules of resiquimod gel.

## 115 **METHODS**

### 116 **TRIAL DESIGN**

117 In a prospective, randomized partly placebo-controlled, double blind (Arms1/2/3), phase II,  
118 dose-finding study, safety, tolerability and efficacy of topical resiquimod gel in patients with  
119 multiple AK was studied. In two additional arms (Arms4/5), patients applied either  
120 resiquimod gel 0.01% or 0.03% 3 times weekly up to a biological end-point. The study was  
121 approved by the Ethics Committees and regulatory authorities (Switzerland and Germany)  
122 and registered at ClinicalTrials.gov (NCT01583816).

### 123 **PATIENTS**

124 Between 5/2014 and 11/2014, 14 sites screened and enrolled patients. Eligible were males  
125 and females over 18 years with  $\geq 2$  clinically (one biopsied) diagnosed AK-lesions (indicator  
126 lesion  $\geq 6\text{mm } \varnothing$ ) within a  $25\text{cm}^2$  contiguous treatment area (balding scalp, forehead, or face),  
127 who gave written informed consent. Patients with unstable significant medical conditions,  
128 active infections, immunosuppression or systemic cancer, autoimmune disorders, HIV,  
129 known thyroid abnormalities, depression, atopic dermatitis, rosacea, eczema, abuse,  
130 allergies or hypersensitivities to any product ingredients, pregnancy or lactation were  
131 excluded. AK treatment related therapy-free time intervals were defined for the time prior to  
132 trial start. Aside from the trial medication no other systemic or topical therapies of AK were  
133 allowed.

### 134 **INTERVENTIONS**

135 Patients self-administered the gel topically to pre-defined treatment areas, according to  
136 dosage and trial schedules (Tab.1). Gel application took place prior to bedtime. Efficacy,  
137 safety and local tolerability of treatments were investigated over  $\leq 24$  weeks. Product dosage  
138 was defined as a string of gel corresponding to  $250\text{mg}$  gel for  $25\text{cm}^2$  i.e.  $1\mu\text{g}/3\mu\text{g}$  resiquimod  
139 per  $\text{cm}^2$ , respectively. Treatment adherence was assessed by collecting and weighing  
140 treatment tubes at each patient visit.

### 141 **OUTCOMES**

142 Efficacy was evaluated based on clinical inspection of treatment area and AK-lesion counts.  
143 Lesions counts at visit1 were compared to lesion counts at trial end. Complete clinical  
144 clearance (CCC) was reached if no previously existing lesion was present at trial end or  
145 after reaching the biological endpoint (manifestation of skin erosion, i.e. reddened, erosive  
146 and weeping skin) plus 8-week follow-up (Arms4/5). Further efficacy endpoints were CCC

at start of the second treatment cycle (Arms1/2/3), disappearance of  $\geq 75\%$  of AK-lesions (PCC; partial clinical clearance) at trial end, histological proof of clearance of the indicator lesion at trial end, global efficacy judgment by investigator and patient by means of point scores (seven-point scale i.e., 1: significantly worse; 2: slightly worse; 3: no change; 4: slightly improved; 5: moderately improved; 6: significantly improved; 7: completely improved) at the starting point of the second treatment cycle (Arms1/2/3) and at trial end (Arms1/2/3/4/5). Quality of life (QoL) was assessed with the Skindex-29 questionnaire at visit2 (baseline), after 1<sup>st</sup> treatment cycle for Arms1/2/3, and after reaching the biological endpoint for Arms4/5, as well at study end<sup>16,17</sup>. The questionnaire encompasses the evaluation of three independent areas - symptoms, emotions, and functioning.

Safety was assessed based on treatment-emerged adverse events (TEAE)/serious AE (SAE), local tolerability (burning, itching, pain) by means of symptom scoring (five-point scale i.e., 0: absent, 1: slight, 2: moderate, 3: severe, 4: very severe), systemic tolerability, blood chemistry, global tolerability judgment by investigator and patient by means of point scores (1-6) at the starting point of the second treatment cycle (Arms1/2/3) and at trial end (Arms1/2/3/4/5) and by the number of withdrawal from the trial. Safety assessments were recorded at each patient visit.

## **SAMPLE SIZE, RANDOMIZATION, BLINDING, STATISTICAL ANALYSIS**

Sample size was determined based on the objective to show superiority of resiquimod over placebo within maximally 24 weeks (chi-square test) related to the primary out-come - CCC. For superiority testing using two-tailed  $\alpha$  of 0.05 (Type-1 error) and  $\beta$  of 0.20 (80% power), efficacy estimates of 25% for placebo (based on literature) and 70% for resiquimod (conservative calculation based on the earlier phase II study<sup>14</sup>), and a 2:1 allocation for resiquimod to placebo, the resulting sample size for comparing one active resiquimod Arm to placebo was 32 resiquimod patients and 16 placebo patients. The total estimated sample size including all five active treatment arms plus the three matched placebos was 208.

Patients meeting eligibility criteria were randomized to one of five treatment arms (Tab.1). In Arms1/2/3, patients were randomly assigned (2:1) to resiquimod gel or placebo. For treatment Arms4/5, parallel group randomization (1:1) was applied. Patients meeting eligibility criteria were assigned to consecutive numbers according to their enrolment and centre. Treatment Arms1/2/3 were controlled each by their corresponding matching placebo (multi-placebo group design) to preserve blinding. Treatment Arms4/5 were mutually blinded with no placebo control. Arms4/5 were compared to placebo of Arm1. A trial independent company prepared trial medication sets and randomly delivered the sets once

181 a patient was announced eligible by a centre. The trial medication set (serially numbered)  
182 with the lowest number was then delivered. Due to the fact that the length of the treatment  
183 was different (Tab.1) some treatment arms may be divined, e.g., Arm3. However, placebo  
184 and vera as well as drug concentration were always blinded. Investigators, assessors, data  
185 analysts and patients remained blinded throughout the study. Identity of resiquimod-  
186 /placebo-treatments and resiquimod concentration was concealed using identical appearing  
187 tubes for all products.  
188



## RESULTS

### PATIENTS

Fourteen study centres participated in this trial. Most patients were enrolled in four study centres (two centres enrolled 39, two enrolled 24 patients).

A total of 290 patients were screened and 218 met eligibility criteria and were randomized. One patient was enrolled but discontinued early and never administered study medication. Thus, 217 Caucasian patients (33 females; 184 males), 43-93 years (mean 70.9-74.1) who received at least one dose of trial medication were included in the Intention-To-Treat (ITT) and in the safety population (identical with ITT-population) analyses (Tab.2). Thirty-seven had major protocol violation(s). Therefore, 180 Caucasian patients (23 females; 157 males), 47-90 years (mean 70.1-74.3) were included in the Per-Protocol (PP) population. Protocol deviations were related to study inclusion or exclusion criteria, safety and efficacy assessments, conduct of trial or patient management. The demographic characteristics of the patients at baseline were similar between study groups (Tab.2). All patients had AK proven by histology. Most patients were of skin photo-type II (n=134), 13 photo-type I, 67 photo-type III and 2 photo-type IV (one missing information).

### OUTCOMES

#### Primary Outcome

In the ITT-population, the overall CCC-rate at study end was 67% (p=0.001 vs placebo), 72% (p=0.004 vs placebo), 70% (p<0.001 vs placebo), 56% (p=0.009 vs placebo), and 74% (p<0.001 vs placebo) for treatment Arms1/2/3/4/5, respectively (Fig.1). The P value in treatment Arms4/5 represents a Chi-Square test for differences between the active treatment Arm and placebo of Arm1. In the PP-population, the overall CCC-rate at study end was 71% (p=0.001 vs placebo), 85% (p<0.001 vs. placebo), 72% (p<0.001 vs. placebo), 56% (p=0.014 vs. placebo) and 79% (p<0.001 vs. placebo) for treatment Arms1/2/3/4/5, respectively (Fig.1).

#### Secondary Outcomes

In the ITT-population, the CCC-rate at the starting point of the second treatment cycle (Arms1/2/3) was 52% (p<0.001 vs placebo), 55% (p=0.002 vs placebo), and 52% (p=0.001 vs placebo) for treatment Arms1/2/3, respectively (Fig.2). In the PP-population, 62% (p<0.001 vs placebo), 63% (p<0.001 vs placebo) and 55% (p=0.002 vs placebo) of patients

220 treated with resiquimod gel showed complete clinical clearance after the first treatment cycle  
221 in treatment Arms1/2/3, respectively (Fig.2).

222 In the ITT-population, PCC of AK-lesions ( $\geq 75\%$ ) was observed in 87% ( $p < 0.001$  vs  
223 placebo), 81% ( $p = 0.004$  vs placebo), 77% ( $p < 0.001$  vs placebo), 75% ( $p = 0.002$  vs placebo)  
224 and 78% ( $p = 0.001$  vs placebo) of patients of Arms1/2/3/4/5, respectively. In the PP-  
225 population 90% ( $p < 0.001$  vs placebo), 89% ( $p < 0.001$  vs placebo), 79% ( $p < 0.001$  vs  
226 placebo), 74% ( $p = 0.004$  vs placebo), and 83% ( $p < 0.001$  vs placebo) of patients treated with  
227 resiquimod gel (Arms1/2/3/4/5) showed PCC at study end (Fig.3).

228 Histological proof of clearance at study end in the ITT-data set has shown that significantly  
229 more patients randomized to active drug were clear in comparison to placebo (of Arm1) in  
230 Arms 1 (67% vs. 13%,  $p < 0.001$ ), 4 (66% vs. 13%,  $p < 0.001$ ), and 5 (76% vs. 13%,  $p < 0.001$ ).  
231 In treatment Arms2/3 the difference to placebo was not significant (Arm2: 58% vs. 25%,  
232  $p = 0.031$ , Arm3: 69% vs. 40%,  $p = 0.067$ ). In the PP-population, 63% (Arm2) to 77% (Arm5)  
233 of patients who administered resiquimod gel showed histological proof of clearance of the  
234 indicator lesion. Results were significantly different from placebo (of Arm1) in Arm1 (65%  
235 vs. 13%,  $p = 0.002$ ), Arm4 (65% vs. 13%,  $p < 0.001$ ) and Arm5 (77% vs. 13%,  $p < 0.001$ ).

236 Point scores of global judgments of efficacy by investigator were higher in all resiquimod  
237 treatment groups as compared to placebo. At the starting point of the 2<sup>nd</sup> treatment cycle  
238 (i.e. after the 1<sup>st</sup> treatment cycle and an 8-week treatment-free interval), the investigators  
239 judged the efficacy as significantly better (ITT and PP-populations) in the resiquimod  
240 treatment groups of Arms 1/2/3 (ITT:  $p < 0.001$ ,  $p < 0.001$ ,  $p = 0.007$ , PP:  $p < 0.001$ ,  $p < 0.01$ ,  
241  $p = 0.009$ ). The mean point scores improved further at study end in these Arms. Compared  
242 to placebo, the efficacy was rated as “significantly improved” at study end in active groups  
243 of treatment Arms1/2/3 for the ITT and PP-populations ( $p < 0.01$ ). At study end mean scores  
244 of active drug groups (Arms1/2/3/4/5) ranged from 4.6-6.4 in the ITT-population (moderately  
245 ( $p < 0.001$ ) to significantly ( $p < 0.001$ ) improved) and from 6.1-6.3 (significantly ( $p < 0.01$ )  
246 improved) in the PP-population. In the placebo groups the mean scores were 4.5-4.6  
247 (slightly to moderately improved). At the starting point of the 2<sup>nd</sup> treatment cycle,  $\geq 65\%$  of  
248 the AK-lesions were judged as significantly to completely improved (ITT and PP-  
249 populations) by the investigators. At study end  $\geq 80\%$  and  $\geq 79\%$  of AK-lesions were  
250 considered to be significantly or completely improved in both the ITT and PP-population,  
251 respectively.

252 Also, the global judgment of efficacy by patient mean point scores were higher in the active  
253 drug groups than in placebo groups at both the beginning of the 2<sup>nd</sup> treatment cycle and at

study end in all analysed populations. Analogously, the mean point scores of the active drug groups rose at the end of study in treatment Arms1/2/3. Compared to placebo, efficacy was rated as significantly improved ( $p \leq 0.001$ ) at study end in Arms1/2/4/5 of both the ITT and PP-populations. The mean scores at study end ranged from 5.9-6.2 (significantly improved) and from 5.9-6.4 (significantly improved) in the ITT and PP-populations, respectively. In the placebo groups mean scores ranged from 4.4-4.9 (slightly to moderately improved) and from 4.4-4.8 (slightly to moderately improved) in the ITT and PP-populations, respectively. At the starting point of the 2<sup>nd</sup> treatment cycle, patients judged efficacy as significantly improved in all active drug groups. The percentage of patient's global efficacy assessment judging efficacy as significantly to completely improved ranged from 59-78% after the 1<sup>st</sup> treatment cycle and from 73-85% at study end in the ITT-population. In the PP-population, percentages ranged from 59-81% and from 71-92% at both the beginning of the 2<sup>nd</sup> treatment cycle and at study end, respectively.

The QoL assessment (Skindex-29) has shown that in the ITT and PP-population, symptoms' scores improved in all resiquimod treatment groups from baseline to visit 8 or to study end, with the highest improvement compared to baseline seen in Arm3 (mean score reduction -3 ( $p < 0.02$ ) (ITT and PP)). The same was observed regarding emotion scores with the highest, not statistically significant versus placebo, reduction in mean score values in Arm2 (ITT-population: -2.3; PP-population: -2.5) and Arm4 (ITT-population: -2.0; PP-population: -2.5). In the ITT- and PP-population, functioning scores improved in treatment Arm4 by -0.6 ( $p = 0.17$ ) and -0.7 ( $p = 0.26$ ) from baseline to study end, respectively.

Analysis of the treatment effect on the primary efficacy endpoint for possible confounders (gender, age:  $< 65$  years and  $\geq 65$  years, skin photo-type, and patient compliance ( $< 75\%$ ;  $\geq 75\%$ )), did not show statistically significant differences for any of them or for each confounder by treatment interaction term for any of the five active treatments versus placebo.

## SAFETY

Patients of all treatment arms reported TEAEs (Tab.3). TEAE of 128 (59%) patients were considered as related to trial drug (ADR; adverse drug reactions). Within this class, "application site erythema" and "application scab" were the most frequently reported reactions with the highest incidences in treatment Arm5 (erythema: 52%, scab: 32%). Thirteen patients discontinued from trial due to TEAEs – five due to severe local skin reactions.

## DISCUSSION

Objectives of this trial were to define the concentration and dosing schedule of resiquimod gel at which CCC (no AK-lesion according to clinical evaluation in treatment area) occurred (Arms1/2/3), or at which a biological endpoint was achieved with subsequent CCC (Arms4/5).

Efficacy was significantly higher in all resiquimod treatment groups compared to placebo with overall CCC-rates at the end of study ranging from 56% to 74% in the ITT-population. Results of all resiquimod treatment Arms were significantly better than placebo ( $p<0.01$ ). Overall, the results were consistent with the hypothesis of 70% clearance with resiquimod versus 25% with placebo and with results from a previous study<sup>14</sup> assessing resiquimod in AK. Results were confirmed in the PP-population in which, the same overall CCC-rate ranged from 56% to 85%. In the PP-analysis differences to placebo were significant in treatment Arms 1/2/3/5 ( $p<0.01$ ).

Regarding CCC-rates after 1<sup>st</sup> treatment cycle (i.e. 4-week treatment plus 8-week treatment-free period), significantly higher percentages in comparison to placebo were observed in all active treatment groups assessed and in both analysis populations.

Across all treatment groups, investigators and patients assessed the efficacy of resiquimod higher than the efficacy of placebo. The mean score values of global judgment of efficacy by the investigators at study end were approximately 6 (significantly improved) with resiquimod and approximately 4.5 (slightly to moderately improved) with placebo. QoL (Skindex-29) symptoms' and emotions' scores improved from baseline to study end in all resiquimod groups supporting the subjective efficacy of the product.

Regarding safety, a higher percentage of resiquimod treated patients (77%) reported adverse events such as general disorders and administration site conditions in comparison to placebo (62%). The highest indices of these events (mostly erythema and scab) were reported in treatment Arm5, which had the highest resiquimod-dosing scheme and the highest overall complete clearance (ITT analysis). These events are seen in all therapies with IRM and can be interpreted as typical signs for immunostimulatory therapies. The main reasons for discontinuation were general disorders and administration site conditions. Overall, the local tolerability of the trial medication was acceptable.

The safety profile is consistent with previously completed clinical phase II and III trials including 2100 patients (resiquimod in various indications)<sup>13,14,16,19</sup>. In these trials ADRs were primarily local skin reactions such as erythema, scab, oedema, erosion, ulceration and

321 vesicles. Systemic ADRs included infections (application site pustules, nasopharyngitis,  
322 and application site infection) and nervous system disorders (headache, paresthesia).

323 In the study conducted by Szeimies et al., influenza like symptoms were reported in one  
324 patient (3%) treated with resiquimod 0.03% gel<sup>14</sup>. In this study, three influenza-like side  
325 effects occurred in treatment Arm1/3. The symptoms are possibly related to cytokine  
326 release/induction, an activity associated with the proposed mechanism of action of  
327 resiquimod as an immune response modifier (IRM).

328 One may compare our data with previous investigations related to topical AK-treatment. In  
329 pooled analysis of trials using similar end points and clinical definitions for CCC and PCC,  
330 the rates of clearance were higher for ingenol mebutate (total of 3 applications for AK's on  
331 face or scalp, evaluated at day 57 after trial initiation) than with placebo - 42.2% vs. 3.7%  
332 and 63.9% vs. 7.4%, respectively<sup>20</sup>. For imiquimod 3.75% (up to 42 applications, evaluated  
333 at day 119 after trial initiation), the rates of clearance were also higher than with placebo –  
334 34.0% vs. 5.5% and 53.7% vs. 12.8%, respectively<sup>21</sup>. For 5-fluorouracil 0.5% and salicylic  
335 acid 10% (up to 84 applications, evaluated at day 140 after trial initiation), the rate of CCC  
336 was higher than with placebo – 55.4% vs. 15.1%<sup>22</sup>. In a recent trial a compounded mixture  
337 of 0.005% calcipotriol ointment (Taro Pharmaceuticals) with 5% 5-fluorouracil cream (Taro  
338 Pharmaceuticals) or Vaseline with 5% 5-fluorouracil cream at a 1:1 weight ratio (8  
339 applications, evaluated at day 56 after trial initiation), the rates of CCC and PCC of AK's on  
340 the face were 27.0% vs. 0% and 80% vs. 0%, respectively<sup>23</sup>.

341 For resiquimod Arms1/2/3 (24, 14, 10 applications, evaluated at day 168, 140, 126 after trial  
342 initiation), the rates of CCC and PCC were also higher than with placebo – 71-85% vs. 13-  
343 21% and 79-90% vs. 27-36%, respectively. And for resiquimod Arms4/5 (up to 24  
344 applications, evaluated at day 112 after trial initiation), the rates of these clearances were  
345 56-79% vs. 19% and 74-83% vs. 31%, respectively.

346 Some limitations apply to this investigation. To study the influence of a) drug concentration,  
347 b) dosing schedule, c) the usefulness of a biological endpoint as a marker for treatment  
348 cessation and d) to relate to a previous phase II study with the same resiquimod formulation  
349 (Arm1) the number of patients per arm was small (n=31-38). There was no assignation of  
350 a direct placebo arm to the treatment Arms4/5. The investigation was only focused on AK  
351 treatment of the balding scalp, forehead, and face. The clinical clearance at study end  
352 proved the superiority of verum vs. placebo. However, the histological proof of clearance in  
353 treatment Arm2/3 vs. placebo was not significant. On the one side, this may be explained  
354 by the fact, that the treatment duration of Arm2/3 was shorter than in Arm1 (24 applications

355 vs. 14 applications vs. 10 applications (2 cycles)), on the other hand it emphasises the  
356 importance of the parallel use of clinical and histological means. However, this statement  
357 is based on only one indicator lesion.

358 Overall, resiquimod 0.03% is numerically more effective than the lower concentration of  
359 0.01%. Effectiveness in the 3 placebo-controlled Arms1/2/3 was largely comparable  
360 between the treatment arms with CCC rates achieved with resiquimod compared to  
361 corresponding placebo. However, the clinical response in Arms2/3 was reached with  
362 significantly less gel applications – 14 and 10 applications instead of 24, respectively. In  
363 treatment Arm5 (same resiquimod strength as in treatment Arm1/2/3, treatment cessation  
364 elicited by biological endpoint) the average number of gel applications was 22.2 (median  
365 13).

366 Taking comparable effectiveness of therapeutic regimens with the 0.03% resiquimod gel  
367 formulations, the two lowest dose/regimens/durations evaluated in this study (one treatment  
368 cycle: Arm2/0.03%, 7x/2 weeks and Arm3/0.03%, 5x/1 week) offer an effective topical IRM  
369 therapy with a considerable shorter treatment period compared to current IRM therapies.  
370 The dosing regimens using the biological end-point of erosion proved equally efficacious as  
371 fixed-time regimes promising a novel personalized approach in treating AK.

372

## REFERENCES

1. de Berker D, McGregor JM, Mohd Mustapa MF, Exton LS, Hughes BR. British Association of Dermatologists' guidelines for the care of patients with actinic keratosis 2017. *Br J Dermatol*. 2017;176(1):20-43. doi: 10.1111/bjd.15107
2. Dodds A, Chia A, Shumack S. Actinic keratosis: rationale and management. *Dermatol Ther (Heidelb)*. 2014 Jun;4(1):11-31. doi: 10.1007/s13555-014-0049-y
3. Ulrich M, Drecoll U, Stockfleth E. Emerging drugs for actinic keratosis. *Expert Opin Emerg Drugs*. 2010;15(4):545-55. doi: 10.1517/14728214.2010.507191
4. Fu W, Cockerell CJ. The actinic (solar) keratosis: a 21st-century perspective. *Arch Dermatol*. 2003;139(1):66-70. PMID: 12533168
5. Flohil SC, van der Leest RJ, Dowlathshahi EA, Hofman A, de Vries E, Nijsten T. Prevalence of actinic keratosis and its risk factors in the general population: the Rotterdam Study. *J Invest Dermatol*. 2013;133(8):1971-8. doi: 10.1038/jid.2013.134.
6. Salasche SJ. Epidemiology of actinic keratoses and squamous cell carcinoma. *J Am Acad Dermatol*. 2000;42(1 Pt 2):4-7. PMID: 10607349
7. Padilla RS, Sebastian S, Jiang Z, Nindl I, Larson R. Gene expression patterns of normal human skin, actinic keratosis, and squamous cell carcinoma: a spectrum of disease progression. *Arch Dermatol*. 2010;146(3):288-93. doi: 10.1001/archdermatol.2009.378.
8. Czarnecki D, Meehan CJ, Bruce F, Culjak G. The majority of cutaneous squamous cell carcinomas arise in actinic keratoses. *J Cutan Med Surg*. 2002;6(3):207-9. doi: 10.1177/120347540200600301.
9. Guenther ST, Hurwitz RM, Buckel LJ, Gray HR. Cutaneous squamous cell carcinomas consistently show histologic evidence of in situ changes: a clinicopathologic correlation. *J Am Acad Dermatol*. 1999;41(3 Pt 1):443-8. PMID: 10459120.
10. Mittelbronn MA, Mullins DL, Ramos-Caro FA, Flowers FP. Frequency of pre-existing actinic keratosis in cutaneous squamous cell carcinoma. *Int J Dermatol*. 1998;37(9):677-81. PMID: 9762818.
11. Werner RN, Sammain A, Erdmann R, Hartmann V, Stockfleth E, Nast A. The natural history of actinic keratosis: a systematic review. *Br J Dermatol*. 2013 Sep;169(3):502-18. doi: 10.1111/bjd.12420.

- 403 12. Stockfleth E, Sibbring GC, Alarcon I. New Topical Treatment Options for Actinic  
404 Keratosis: A Systematic Review. *Acta Derm Venereol.* 2016;96(1):17-22. doi:  
405 10.2340/00015555-2167.
- 406 13. Meyer T, Surber C, French LE, Stockfleth E. Resiquimod, a topical drug for viral skin  
407 lesions and skin cancer. *Expert Opin Investig Drugs.* 2013;22(1):149-59. doi:  
408 10.1517/13543784.2013.749236.
- 409 14. Szeimies RM, Bichel J, Ortonne JP, Stockfleth E, Lee J, Meng TC. A phase II dose-  
410 ranging study of topical resiquimod to treat actinic keratosis. *Br J Dermatol.*  
411 2008;159(1):205-10. doi: 10.1111/j.1365-2133.2008.08615.x.
- 412 15. Lee M, Park CS, Lee YR, Im SA, Song S, Lee CK. Resiquimod, a TLR7/8 agonist,  
413 promotes differentiation of myeloid-derived suppressor cells into macrophages and  
414 dendritic cells. *Arch Pharm Res.* 2014;37(9):1234-40. doi: 10.1007/s12272-014-0379-4.
- 415 16. Mark KE, Corey L, Meng TC, Magaret AS, Huang ML, Selke S, Slade HB, Tyring SK,  
416 Warren T, Sacks SL, Leone P, Bergland VA, Wald A. Topical resiquimod 0.01% gel  
417 decreases herpes simplex virus type 2 genital shedding: a randomized, controlled trial.  
418 *J Infect Dis.* 2007;195(9):1324-31. doi: 10.1086/513276.
- 419 17. Augustin M, Wenninger K, Amon U, Schroth MJ, Küster W, Chren M, Kupfer J, Gieler  
420 U. German adaptation of the Skindex-29 questionnaire on quality of life in dermatology:  
421 validation and clinical results. *Dermatology.* 2004;209(1):14-20. doi:  
422 10.1159/000078581
- 423 18. Rook AH, Gelfand JM, Wysocka M, Troxel AB, Benoit B, Surber C, Elenitsas R,  
424 Buchanan MA, Leahy DS, Watanabe R, Kirsch IR, Kim EJ, Clark RA. Topical resiquimod  
425 can induce disease regression and enhance T-cell effector functions in cutaneous T-cell  
426 lymphoma. *Blood.* 2015;126(12):1452-61. doi: 10.1182/blood-2015-02-630335.
- 427 19. Aranda F, Vacchelli E, Obrist F, Eggermont A, Galon J, Sautès-Fridman C, Cremer I,  
428 Henrik Ter Meulen J, Zitvogel L, Kroemer G, Galluzzi L. Trial Watch: Toll-like receptor  
429 agonists in oncological indications. *Oncoimmunology.* 2014;3:e29179. eCollection  
430 2014. PMID: 25083332.
- 431 20. Lebwohl M, Swanson N, Anderson LL, Melgaard A, Xu Z, Berman B. Ingenol mebutate  
432 gel for actinic keratosis. *N Engl J Med.* 2012;366(11):1010-9. doi:  
433 10.1056/NEJMoa1111170.



- 434 21. Hanke CW, Beer KR, Stockfleth E, Wu J, Rosen T, Levy S. Imiquimod 2.5% and 3.75%  
435 for the treatment of actinic keratoses: results of two placebo-controlled studies of daily  
436 application to the face and balding scalp for two 3-week cycles. *J Am Acad Dermatol.*  
437 2010;62(4):573-81. doi: 10.1016/j.jaad.2009.06.020.
- 438 22. Stockfleth E, Kerl H, Zwingers T, Willers C. Low-dose 5-fluorouracil in combination with  
439 salicylic acid as a new lesion-directed option to treat topically actinic keratoses:  
440 histological and clinical study results. *Br J Dermatol.* 2011;165(5):1101-8. doi:  
441 10.1111/j.1365-2133.2011.10387.x.
- 442 23. Cunningham TJ, Tabacchi M, Eliane JP, Tuchayi SM, Manivasagam S, Mirzaalian H,  
443 Turkoz A, Kopan R, Schaffer A, Saavedra AP, Wallendorf M, Cornelius LA, Demehri S.  
444 Randomized trial of calcipotriol combined with 5-fluorouracil for skin cancer precursor  
445 immunotherapy. *J Clin Invest.* 2017;127(1):106-116. doi: 10.1172/JCI89820.

**Table 1: Trial Design, Interventions, Dosage and Trial Schedule**

Treatment Arm	Strength		Treatment and Follow-Up Phases																							
Week No.		-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Visits		v <sub>1</sub> *	v <sub>2</sub> *			v				v					v			v				v				v
Arm1	0.03%		3x*	3x	3x	3x	8-week break								3x	3x	3x	3x	8-week break							
Placebo			3x	3x	3x	3x	8-week break								3x	3x	3x	3x	8-week break							
			1 <sup>st</sup> Treatment Cycle												2 <sup>nd</sup> Treatment Cycle											

Week No.		-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Visits		v <sub>1</sub>	v <sub>2</sub>	v				v					v	v				v				v
Arm2	0.03%		7x*	8-week break								7x	8-week break									
Placebo			7x	8-week break								7x	8-week break									
			1 <sup>st</sup> Treatment Cycle										2 <sup>nd</sup> Treatment Cycle									

Week No.		-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Visits		v <sub>1</sub>	v <sub>2</sub>	V			v					vv				v				v
Arm3	0.03%		5x*	8-week break								5x	8-week break							
Placebo			5x	8-week break								5x	8-week break							
			1 <sup>st</sup> Treatment Cycle									2 <sup>nd</sup> Treatment Cycle								

Week No.		-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Visits		v <sub>1</sub>	v <sub>2</sub>	v	v	v	v	v	v	v								v
Arm4	0.01%		3x	3x	3x	3x	3x	3x	3x	3x	8-week break							
Arm5	0.03%		3x	3x	3x	3x	3x	3x	3x	3x	8-week break							

\*v: visit, v<sub>1</sub>: screening visit, v<sub>2</sub>: baseline visit, 3x: applications on Mondays, Wednesdays and Fridays, 7x: applications on Mondays, Wednesdays, Fridays, Sundays, Tuesdays, Thursdays and Saturdays, 5x: applications on Mondays, Tuesdays, Wednesdays, Thursdays and Fridays,

**Table 2: Demographic Characteristics of the Patients at Baseline. \***

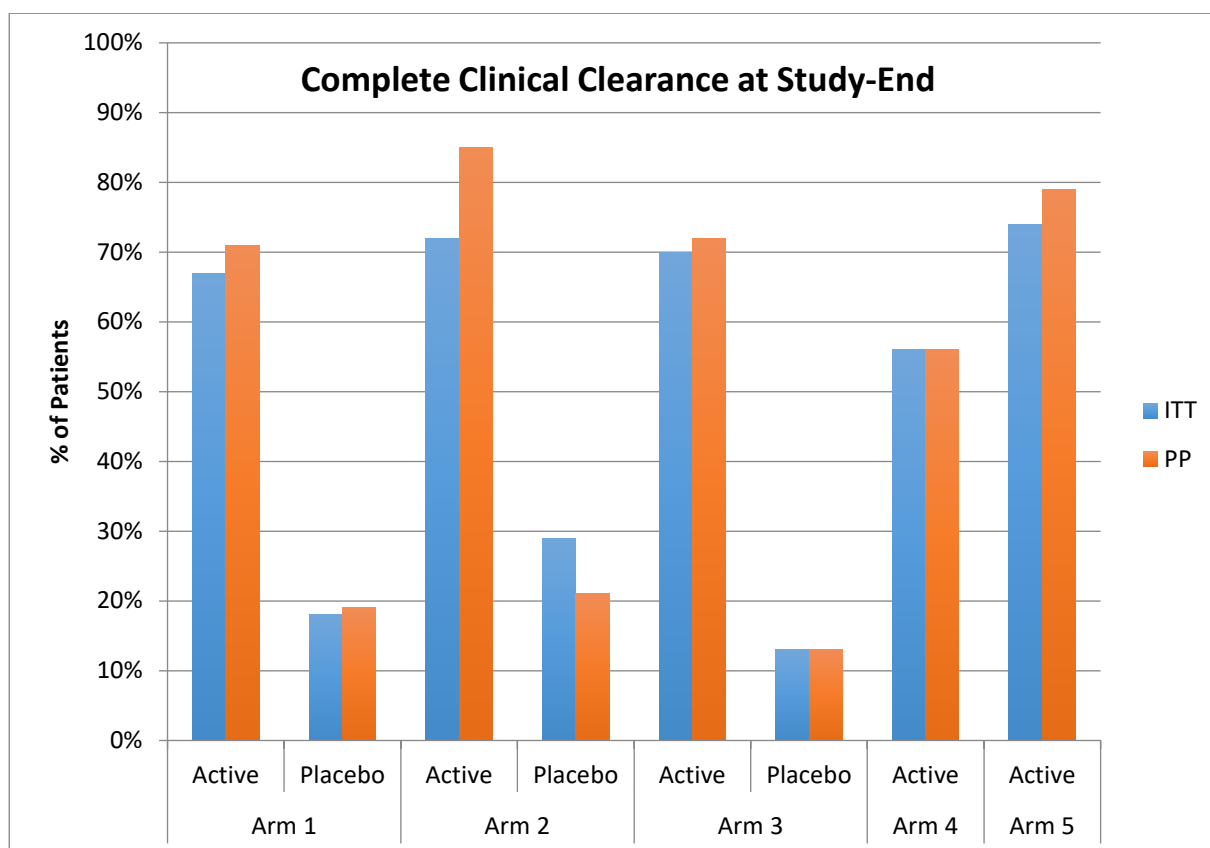
ITT- Safety Population								
	Arm 1		Arm 2		Arm 3		Arm 4	Arm 5
	Resiquimod (N=31)	Placebo (N=17)	Resiquimod (N=33)	Placebo (N=18)	Resiquimod (N=31)	Placebo (N=18)	Resiquimod (N=38)	Resiquimod (N=31)
Age-yr*	73.9±6.9	73.9±10.1	72.8±8.1	71.3±8	72.7±6.9	72.3±9.1	70.9±8.7	74.1±9.5
Male sex – no. (%)	25 (81)	14 (82)	29 (88)	16 (89)	26 (84)	15 (83)	34 (89)	25 (81)
PP- Efficacy Population								
	(N=21)	(N=16)	(N=27)	(N=14)	(N=29)	(N=15)	(N=34)	(N=24)
Age-yr*	73.1±6.1	73.6±10.4	71.9±7.9	72.2±7.2	72.5±7	74.3±4	70.1±8.6	73.8±9.1
Male sex – no. (%)	20 (95)	13 (81)	23 (85)	13 (93)	24 (83)	13 (87)	31 (91)	20 (83)

\* Plus-minus values are means ±SD.

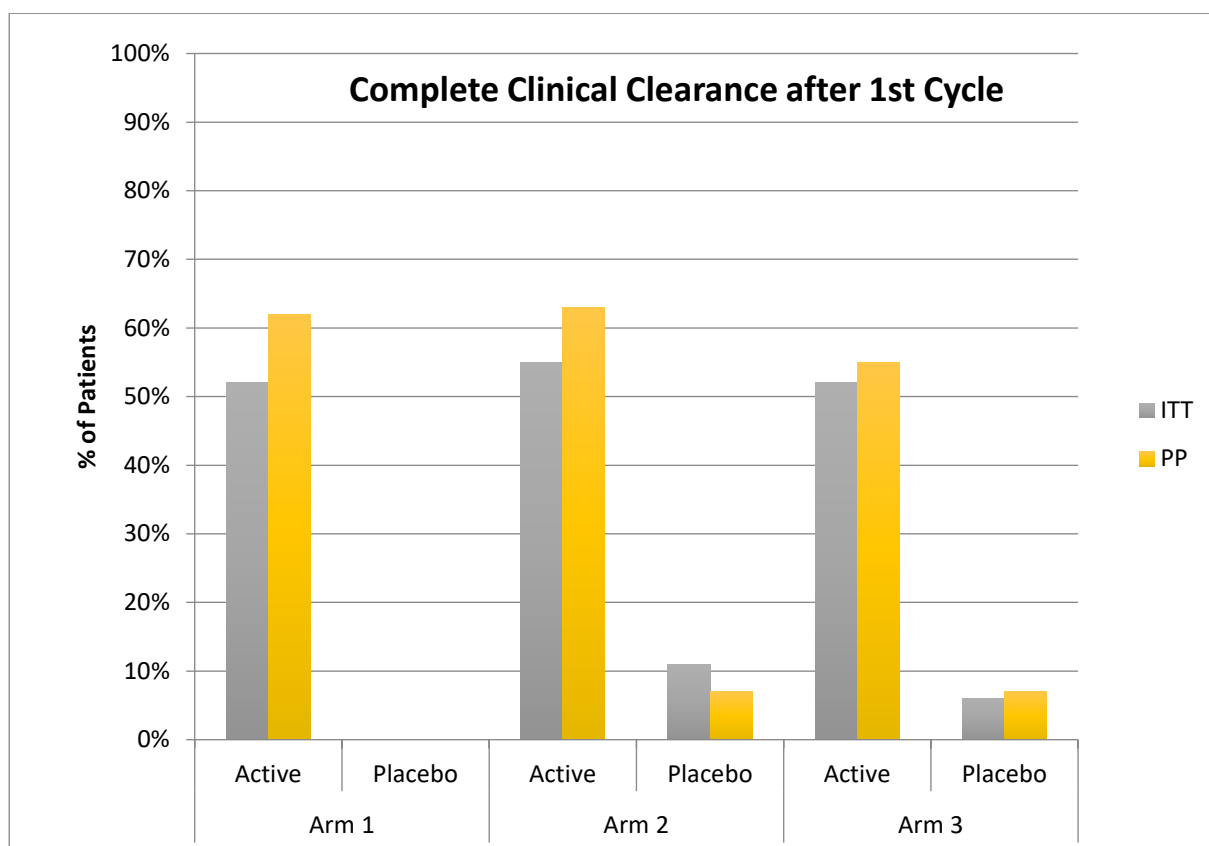
**Table 3: Overview of Treatment-Emergent Adverse Events**

	Arm 1		Arm 2		Arm 3		Arm 4	Arm 5
	Resiquimod (N=31)	Placebo (N=17)	Resiquimod (N=33)	Placebo (N=18)	Resiquimod (N=31)	Placebo (N=18)	Resiquimod (N=38)	Resiquimod (N=31)
TEAEs	22 (71%)	11 (65%)	23 (70%)	11 (61%)	24 (77%)	11 (61%)	32 (84%)	26 (84%)
SAEs	1 (3%)	1 (6%)	4 (12%)	3 (17%)	0	0	0	1 (3%)
Discontinuations due to AEs	7 (23%)	0	0	0	0	0	2 (5%)	4 (13%)
ADRs	20 (65%)	3 (18%)	18 (55%)	6 (33%)	20 (65%)	5 (28%)	30 (79%)	26 (84%)
SADRs	0	0	0	0	0	0	0	0
Deaths	0	0	0	0	0	0	0	0

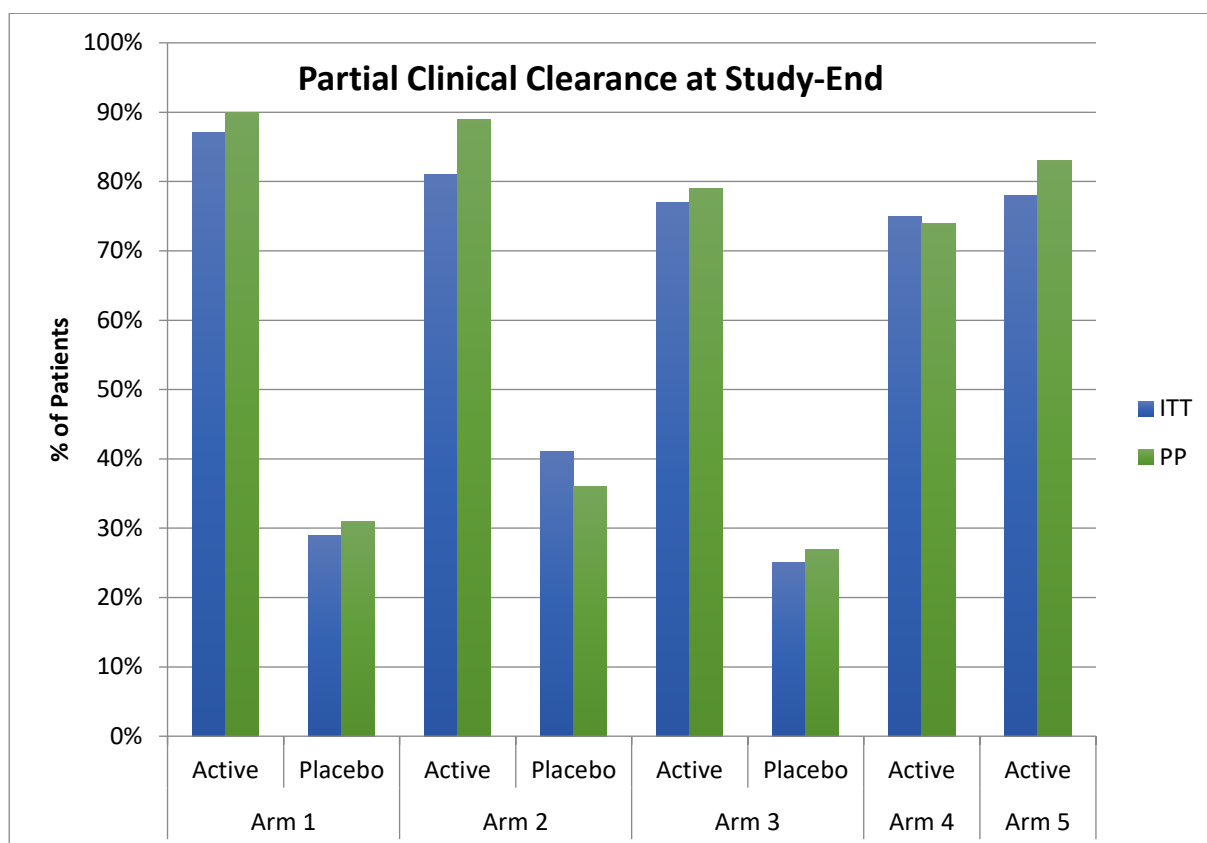
TEAE: Treatment-Emergent Adverse Event, SAE: Serious Adverse Event, ADR: Adverse Drug Reaction, SADR: Serious Adverse Drug Reaction



**Figure 1.** Percentage of patients (ITT and PP population) showing complete clinical clearance at study-end.



**Figure 2:** Secondary efficacy results (ITT- and PP population) - complete clinical clearance after 1<sup>st</sup> cycle.



**Figure 3:** Secondary efficacy results (ITT- and PP population) - Partial clinical clearance of AK lesions ( $\geq 75\%$ ).